REMARKS

Favorable reconsideration of this application in light of the preceding amendments and the following elections and remarks is requested. Initially, Applicant notes that the previous Response to Restriction Requirement included new Claims 13-19. Claim 19 was drawn to a method of producing a transgenic fish. Applicant clarifies that prior to the amendment submitted herein, Claims 1-19 were properly pending. The present Claim Amendment has resulted in the cancellation of Claims 2-19, an amendment of Claim 1 and the addition of Claim 20. Support for the Claim amendment and for the new Claim exists throughout the specification, at least on pages 2-3, lines 24-28 spanning from the bottom of page 2 to line 5 of page 3, page 4, lines 8-17 and 25-29, and page 5, lines 1-9.

SPECIFICATION OBJECTION

The Applicant has amended the Application in accordance with proper U.S. patent practice. Applicant respectfully submits that the objections to the Specification have been overcome by the present amendment.

CLAIM OBJECTIONS

Claim 1 is objected to because of the following informalities: Claim 1 refers to "a fluorescent gene".

The Applicant has amended Claim 1 to recite a "red-fluorescence protein reporter gene" and "green fluorescent protein reporter gene" thus obviating the Examiner's noted objection.

The Examiner recites that Claims 15-17 are objected to because "Genes are not, themselves, fluorescent. However, the products they encode can be." The Applicant has cancelled Claims 2-19, thus rendering this objection moot.

Accordingly, the Applicant respectfully requests the Examiner to reconsider and withdraw the present objection.

REJECTION UNDER 35 U.S.C. § 112-2ND PARAGRAPH

Claims 1-4 and 14-17 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point and distinctly claim the subject matter which Applicant regards as the invention. This rejection is respectfully traversed.

The Applicant respectfully disagrees with the Action's alleged conclusion that "ubiquitous promoter" is unclear and indefinite given the knowledge of those skilled in the art at the time of filing and the numerous and concrete examples provided in the specification, for example on pages 4-5. The term "ubiquitous promoter" is generally known in the art as a promoter that can be used to express any number of heterologous or homologous genes in an several tissues and organisms. Ubiquitous promoter can generally mean a promoter that can function in many different tissues, and/or organisms as opposed to a tissue-specific promoter.

Similarly, tissue specific promoters can include promoters that are generally functional in a tissue type or similarly related tissue types. If the tissue-specific promoter were able to drive gene expression "in any tissue or multiple tissues" (Action, page 4) they would not be considered by one of ordinary skill as tissue-specific promoters. The Applicant fails to see why after providing several examples of ubiquitous promoters and tissue-specific promoters for use in Zebrafish, the inclusion of the terms renders Claim 1 undefined and not clear. Applicant thus

reserves the right to reintroduce claims reciting "ubiquitous promoters" and "tissue-specific promoters" in a continuing application, while pursuing claims as amended herein in the interest of advancing prosecution of the present application by reducing the number of issues outstanding.

Claim 1 has been amended to recite: A recombinant plasmid, comprising: (a) a α-actin promoter operably linked to a red-fluorescence protein reporter gene; and (b) a β-actin promoter operably linked to a green-fluorescent protein reporter gene; wherein the α-actin promoter and the β-actin promoter have an adverse directional property and are located upstream of the red-fluorescence protein reporter gene and green-fluorescent protein reporter gene respectively permitting directional transcription of each of the reporter genes.

The Applicant submits that the amended claim 1, reciting a β-actin promoter and a α-actin promoter therefore obviates the present rejection. Support for such an amendment to Claim 1 can be found for example on page 4, lines 29 – page 5, lines 2 and on page 5, lines 3-9.

Claims 2-19 have been cancelled herein, thus rendering the present rejection as to Claims 2-4, and 14-17 moot.

New Claim 20 is similarly drawn to a recombinant plasmid comprising an alpha-actin (α-actin) and a beta-actin (β-actin) promoter as discussed above for Claim 1 and should similarly be allowable.

Applicant respectfully submits that the present amendments have clarified the claimed subject matter and obviates the present rejection under 35 U.S.C. § 112- 2nd Paragraph. Accordingly, the Applicant respectfully requests the Examiner to reconsider and withdraw the present rejection under 35 U.S.C. § 112, second paragraph.

REJECTION UNDER 35 U.S.C. § 103

Claims 1, 2, 4 and 15-17 stand rejected under 35 U.S.C. § 103(a) as being anticipated by Yu et al. (November 2002, Blood, Vol. 100, Abstract #1698)) herein referred to as "Yu" in view of Flanagan (1987, Virus Genes, 1:61-71) herein referred to as "Flanagan". This rejection is respectfully traversed.

Claim 1 is drawn to a recombinant plasmid, comprising: (a) a α-actin promoter operably linked to a red-fluorescence protein reporter gene; and (b) a β-actin promoter operably linked to a green-fluorescent protein reporter gene; wherein the α-actin promoter and the β-actin promoter have an adverse directional property and are located upstream of the red-fluorescence protein reporter gene and green-fluorescent protein reporter gene respectively permitting directional transcription of each of the reporter genes. The promoters are on separate strands of the plasmid's double stranded polynucleotide sequence. Thus, the position of the promoters in the different strands enables the promoters to be directionally adverse to one another.

The Examiner states that "Yu et al report that the use of separate, unique promoters, to drive expression of each desired reporter gene resulted in the expression of both reporter genes. Yu et al. taught the use of constitutive promoters including EF-1α (claim 2) and CMV operably linked to GFP and RFP, respectively...Yu et al. used tissue-specific promoters as well as including MHC-II-specific HLA-Drα, in combination with a constitutive promoter." (Action, page 5) Further the Action states that the missing limitation i.e. "the respective orientations of each transgene within the vector" is not discussed by Yu et al. (Action, page 6). This limitation is allegedly provided by Flanagan. The Action alleges that Flanagan teaches the use of plasmid to express two different reporter genes in opposing direction driven by two different promoters. (Action, page 6).

Contrary to the Action's assertion: "One of skill in the art at the time of filing would have been motivated to combine the teachings of Yu et al. relating to a plasmid comprising two independent genes wherein each gene comprises a gene encoding a fluorescent protein operably linked to different promoter, with one promoter being a constitutive promoter and the other being a tissue-specific promoter, with those of Flanagan teaching that divergent orientation of two promoters within a single plasmid construct reduces promoter interference. (Action, page 6), the Applicant respectfully submits that Yu et al, does not disclose a plasmid at all, but rather discloses lentiviral vectors for non-replicating cells. Secondly, Flanagan teaches the use of promoters that are viral specific promoters which are not examples of β-actin and α-actin promoters or equivalents thereof. Furthermore, Flanagan fails to disclose, teach or suggest the use of β-actin and α-actin promoters to drive the expression of green fluorescent protein and red fluorescent protein when used in divergent orientations as described by the present invention.

None of the references cited in the Office Action dated 03/07/2007 disclose, teach or suggest the use of β-actin and α-actin promoters in divergent orientations in any plasmid to produce the red and green fluorescent proteins in any organism. Yu describes the use of lentiviral vectors having a pair of transgenes, GFP driven by EF-1α and RFP driven by CMV promoter in human cells. Flanagan describes experiments associated with expression of two proteins chloramphenicol acetyltransferase (CAT) and β-galactosidase (β-gal) using 3 different activatable HSV promoters.

The Applicant distinguishes the presently cited references of record from the present invention on several grounds. Firstly, in both Yu et al. and in Flanagan, the expression vectors were used for transient expression of the transgene and not for stable transgenic expression in

zebrafish, which would be quite unexpected from the data and teachings of Yu and Flanagan. For example, Flanagan states: "The description and manipulation of the factors involved in the differential activation and repression of various viral transcription units would be greatly aided with development of transient expression constructs that would allow the simultaneous study of two promoters of different kinetic classes.") (Flanagan, page 62, 1st par.). Clearly, Flanagan teaches transient expression of the transgenes and does not teach or suggest a plasmid having non-viral promoters such as β-actin, and α-actin promoters, in divergent orientations.

Secondly, Yu provides a cursory description of using lentiviral vectors and not recombinant plasmids to produce expression of a transgene. Moreover, the lentiviral vectors used in Yu, typically contain several other vector elements that enhance viral transduction of mammalian cells including LTRs, ITRs, U6-shRNA, RRE and other viral specific vector elements. The experiments of Yu were also designed to transfect cells with vectors at high multiplicity of infection. Therefore, one of ordinary skill in the art would not expect the plasmids described in the present invention to function like the lentiviral vectors of Yu and expect the plasmids to produce the fluorescent proteins described in the prior art references.

Thirdly, the recombinant plasmids of the present invention utilize dual promoter sequences, one a ubiquitous promoter like β-actin, and the second a α-actin promoter, oriented in a divergent orientation with respect to one another. Flanagan did not disclose, teach or suggest the use of a α-actin promoter and a β-actin promoter in any construct as disclosed in the present invention. Flanagan's promoters were all Herpes Simplex Virus -1 promoters (Flanagan, page 65). In addition, Flanagan did not use β-actin and α-actin promoters for stable transgenic production of multiple fluorescent reporter protein in any of the constructs. Moreover, the plasmids described by Flanagan did not incorporate fluorescent protein reporter

genes as presently recited in the above amended claims. Claims 2, 4 and 15-17 have been presently cancelled thus rendering this rejection moot as to these claims.

Claims 3 and 14 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yu et al. in view of Flanagan et al. as applied to claims 1, 2, 4 and 15-17 above, and further in view of Ju et al. (1999, Development Genetics, 25:158-167) or Higashijima (1997, Developmental Biology, 192:289-299).

Applicant respectfully submits that the amended claims herein render this rejection moot by virtue of the cancellation of Claims 2-19.

Accordingly, the Applicant respectfully requests the Examiner to reconsider and withdraw the present rejections under 35 U.S.C. § 103(a) of Claims 1-4 and 5-17

CONCLUSION

Accordingly, in view of the above amendments and remarks, reconsideration of the objections and rejections and allowance of each of claims 1 and 20 in connection with the present application are earnestly solicited.

Pursuant to 37 C.F.R. §§ 1.17 and 1.136(a), Applicant(s) hereby petition(s) for a three (3) month extension of time for filing a reply to the outstanding Office Action and submit the required $510.00 extension fee herewith.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact John A. Castellano at the telephone number of the undersigned below.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 08-0750 for any

additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

HARNESS, DICKEY, & PIERCE, P.L.C.

By _____

John A. Castellano, Reg. No. 35,094

P.O. Box 8910
Reston, Virginia 20195
(703) 668-8000

JAC/FEA